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☐ 1. Document ID: US 5869258 A

L7: Entry 1 of 6 File: USPT Feb 9, 1999

US-PAT-NO: 5869258
DOCUMENT-IDENTIFIER: US 5869258 A

TITLE: Detection system for mutagens that identifies mutagenic changes

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gee; Pauline	Berkeley	CA		
Maron; Dorothy M.	Orinda	CA		
Ames; Bruce N.	Berkeley	CA		

US-CL-CURRENT: 435/6; 435/252.3, 435/252.8, 435/29

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 2. Document ID: US 5712386 A

L7: Entry 2 of 6 File: USPT Jan 27, 1998

US-PAT-NO: 5712386
DOCUMENT-IDENTIFIER: US 5712386 A

TITLE: Kits for detecting a target nucleic acid with blocking oligonucleotides

DATE-ISSUED: January 27, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Chang-Ning J.	Chelmsford	MA		
Wu; Kai-Yuan	Lowell	MA		

US-CL-CURRENT: 536/24.33; 435/6, 435/91.2, 536/23.1, 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 3. Document ID: US 5681737 A

L7: Entry 3 of 6 File: USPT Oct 28, 1997

US-PAT-NO: 5681737
DOCUMENT-IDENTIFIER: US 5681737 A

TITLE: Detection system for mutagens that also identifies mutagenic changes

DATE-ISSUED: October 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gee; Pauline	Berkeley	CA		
Maron; Dorothy M.	Orinda	CA		
Ames; Bruce N.	Berkeley	CA		

US-CL-CURRENT: 435/252.3; 435/252.8, 435/320.1, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw. Desc | Image

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☐ 4. Document ID: US 5627054 A

L7: Entry 4 of 6 File: USPT May 6, 1997

US-PAT-NO: 5627054
DOCUMENT-IDENTIFIER: US 5627054 A

TITLE: Competitor primer asymmetric polymerase chain reaction

DATE-ISSUED: May 6, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gillespie, deceased; David	late of Glenmore	PA		

US-CL-CURRENT: 435/91.2; 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw. Desc | Image

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☐ 5. Document ID: US 5567583 A

L7: Entry 5 of 6 File: USPT Oct 22, 1996

US-PAT-NO: 5567583
DOCUMENT-IDENTIFIER: US 5567583 A

TITLE: Methods for reducing non-specific priming in DNA detection

DATE-ISSUED: October 22, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wang; Chang-Ning J.	Chelmsford	MA		
Wu; Kai-Yuan	Lowell	MA		

US-CL-CURRENT: 435/6; 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference KWMC | Draw. Desc | Image

☐ 6. Document ID: US 5075216 A

L7: Entry 6 of 6 File: USPT Dec 24, 1991

US-PAT-NO: 5075216
DOCUMENT-IDENTIFIER: US 5075216 A

TITLE: Methods for DNA sequencing with thermus aquaticus DNA polymerase

DATE-ISSUED: December 24, 1991

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Innis; Michael A.	Moraga	CA		
Myambo; Kenneth B.	Pittsburg	CA		
Gelfand; David H.	Oakland	CA		
Brow; Mary Ann D.	Oakland	CA		

US-CL-CURRENT: 435/6; 435/5, 435/810, 435/91.2, 436/501, 436/808, 536/24.33, 536/26.26, 536/27.14, 536/28.2

Full | Title | Citation | Front | Review | Classification | Date | Reference KWMC | Draw. Desc | Image

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LABECK.DWPI,EPAB,JPAB,USPT.	1
LABECO.DWPI,EPAB,JPAB,USPT.	44
LABED.DWPI,EPAB,JPAB,USPT.	10
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09/345,189

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 NEWS 16  Oct 15    Calculated properties now in the REGISTRY/ZREGISTRY File
 NEWS 17  Oct 22    Over 1 million reactions added to CASREACT
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=> s fluorescence correlation spectroscopy and (asymmetric PCR or asymmetric amplifications)
L1 5 FLUORESCENCE CORRELATION SPECTROSCOPY AND (ASYMMETRIC PCR OR ASYMMETRIC AMPLIFICATIONS)

=> s l1 and ratio#
L2 0 L1 AND RATIO#

=> s l1 and (label## or mark## or dye##)
L3 5 L1 AND (LABEL## OR MARK## OR DYE##)

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PROCESSING COMPLETED FOR L3
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L4 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 2000:408543 CAPLUS
DN 133:40268
TI Method for quantitative analysis of the target nucleic acid by **asymmetric PCR** and **fluorescence correlation spectroscopy**
IN Kinjo, Seikou; Kaneki, Masataka
PA Japan
SO Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2000166599	A2	20000620	JP 1998-353254	19981211

AB A method for quant. anal. of the target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy** (FCS), without a need for sepn. of free and bound **label**, is disclosed. Nucleic acid amplification is carried out with a pair of primers, one **labeled** and the other unlabeled. The **label** is preferably a fluorescent **label**, and unlabeled primers are in excess. The method also comprises the steps of obtaining multiple data measurement within a limited 3D visual field on the soln. mobility/fluctuation of the **label**, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The limited 3D visual field is diffraction limited range formed by confocal optics. Single photon measurement mode is used. The method was applied to the quant. anal. of lambda phage DNA using a rhodamine

labeled primers.

TI Method for quantitative analysis of the target nucleic acid by **asymmetric PCR** and **fluorescence correlation spectroscopy**

AB A method for quant. anal. of the target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy** (FCS), without a need for sepn. of free and bound **label**, is disclosed. Nucleic acid amplification is carried out with a pair of primers, one **labeled** and the other unlabeled. The **label** is preferably a fluorescent **label**, and unlabeled primers are in excess. The method also comprises the steps of obtaining multiple data measurement within a limited 3D visual field on the soln. mobility/fluctuation of the **label**, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The limited 3D visual field is diffraction limited range formed by confocal optics. Single photon measurement mode is used. The method was applied to the quant. anal. of lambda phage DNA using a rhodamine **labeled** primers.

ST nucleic acid quantification arithmetic statistics microscope; DNA amplification asym PCR **fluorescence correlation spectroscopy**

IT PCR (polymerase chain reaction)
(asym.; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Fluorometry
(correlation, (FCS); method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Mathematical methods
(curve, for dynamics of the **label**, anal. of; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**
)

IT Primers (nucleic acid)
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(**labeled**; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Algorithm
Autocorrelation function
Fluorescent indicators
Fluorescent probes
Microscopes
Nucleic acid amplification (method)
Statistical analysis
(method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Nucleic acids
RL: ANT (Analyte); ANST (Analytical study)
(method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Primers (nucleic acid)
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT Diffusion
(of **label** in soln., measurement of; method and app. for

quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT 146368-16-3, Cy3
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(Cy3; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT 146368-14-1, Cy5
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(Cy5; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

IT 81-88-9 13558-31-1 27072-45-3, FITC 47165-04-8, DAPI 169799-14-8, Cy7 172777-84-3, Cy5.5 189767-45-1, Cy3.5
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and **fluorescence correlation spectroscopy**)

L4 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 2000:275403 CAPLUS
DN 132:304266
TI Quantitative determination of target nucleic acid by **asymmetric PCR** and **fluorescence correlation spectroscopy**
IN Kinjo, Masataka
PA Japan
SO Ger. Offen., 18 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 19950823	A1	20000427	DE 1999-19950823	19991021
	JP 2000125900	A2	20000509	JP 1998-301316	19981022
PRAI	JP 1998-301316		19981022		

AB The invention concerns a quant. procedure for target nucleic acid detection using a amplification reaction and a **labeled** primer. No sepn. of free and hybridized **labeled** DNA is necessary. Thus, a PCR reaction using a non-tagged first primer and a fluorescence-tagged second primer, present in fewer copies than the first primer, is carried out. The reaction may be followed by **fluorescence correlation spectroscopy**. These results are statistically analyzed and used to det. the no. of target nucleic acids in the sample.

TI Quantitative determination of target nucleic acid by **asymmetric PCR** and **fluorescence correlation spectroscopy**
AB The invention concerns a quant. procedure for target nucleic acid detection using a amplification reaction and a **labeled** primer. No sepn. of free and hybridized **labeled** DNA is necessary. Thus, a PCR reaction using a non-tagged first primer and a fluorescence-tagged second primer, present in fewer copies than the first primer, is carried out. The reaction may be followed by **fluorescence correlation spectroscopy**. These results are statistically analyzed and used to det. the no. of target nucleic acids in the sample.

ST asym PCR **fluorescence correlation spectroscopy** DNA quantitation

IT PCR (polymerase chain reaction)

(asym.; quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

IT Fluorometry
(correlation; quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

IT Primers (nucleic acid)
RL: ARG (Analytical reagent use); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(fluorophore-**labeled**; quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

IT DNA
RL: ANT (Analyte); ANST (Analytical study)
(quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

IT 13558-31-1D, conjugates with primers 27072-45-3D, FITC, conjugates with primers 47165-04-8D, DAPI, conjugates with primers 146397-20-8D, Cy3, conjugates with primers 169799-14-8D, Cy7, conjugates with primers 172777-84-3D, Cy5.5, conjugates with primers 189767-45-1D, Cy3.5, conjugates with primers
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

IT 264902-73-0, 1: PN: DE19950823 SEQID: 8 unclaimed DNA 264902-74-1, 2: PN: DE19950823 SEQID: 9 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; quant. detn. of target nucleic acid by asym. PCR and **fluorescence correlation spectroscopy**)

L4 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
AN 1998:490523 BIOSIS
DN PREV199800490523
TI Detection of **asymmetric PCR** products in homogeneous solution by **fluorescence correlation spectroscopy**.
AU Kinjo, Masataka (1)
CS (1) Lab. Biophysics, Res. Inst. Electronic Sci., Hokkaido Univ., 060 Sapporo Japan
SO Biotechniques, (Oct., 1998) Vol. 25, No. 4, pp. 706-715.
ISSN: 0736-6205.
DT Article
LA English
AB The yield of the double-stranded DNA product (500 bp) of **asymmetric PCR** with a rhodamine-**labeled** primer (Rho-primer) was determined in a homogeneous solution using **fluorescence correlation spectroscopy** (FCS). FCS provides the average number of molecules in a focused volume and the diffusion constant that relates the molecular weight. Since FCS measures the fluctuation of fluorescence intensity in a very small sample volume, the reaction mixture was directly placed on the FCS optical field without any purification procedure after amplification. The result of changing the initial number of templates suggested that elongation of the Rho-primer could be detected by FCS in a PCR mixture containing a single copy of the target gene in the initial condition. Possible scientific applications and perspectives of the proposed approach are discussed.
TI Detection of **asymmetric PCR** products in homogeneous solution by **fluorescence correlation spectroscopy**.
AB The yield of the double-stranded DNA product (500 bp) of **asymmetric PCR** with a rhodamine-**labeled** primer (Rho-primer) was determined in a homogeneous solution using **fluorescence correlation spectroscopy** (FCS). FCS provides the average number of molecules in a focused volume and the

Can't use it if the date has to be one year of they are the same ~~not~~ inventor

diffusion constant that relates the molecular. . .

IT Methods & Equipment

fluorescence correlation spectroscopy:
analytical method, spectroscopic techniques: CB; PCR [polymerase chain reaction]: DNA amplification, in-situ recombinant gene expression detection, molecular genetic method, sequencing. . .

=>

L5 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS
AN 2000:408543 CAPLUS
DN 133:40268
TI Method for quantitative analysis of the target nucleic acid by **asymmetric PCR** and fluorescence correlation spectroscopy
IN Kinjo, Seikou; Kaneki, Masataka
PA Japan
SO Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000166599	A2	20000620	JP 1998-353254	19981211

AB A method for quant. anal. of the target nucleic acid by asym. PCR and fluorescence correlation spectroscopy (FCS), without a need for sepn. of free and bound label, is disclosed. Nucleic acid amplification is carried out with a pair of **primers**, one **labeled** and the other unlabeled. The **label** is preferably a fluorescent **label**, and unlabeled **primers** are in excess. The method also comprises the steps of obtaining multiple data measurement within a limited 3D visual field on the soln. mobility/fluctuation of the label, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The limited 3D visual field is diffraction limited range formed by confocal optics. Single photon measurement mode is used. The method was applied to the quant. anal. of lambda phage DNA using a rhodamine **labeled primers**.
TI Method for quantitative analysis of the target nucleic acid by **asymmetric PCR** and fluorescence correlation spectroscopy
AB A method for quant. anal. of the target nucleic acid by asym. PCR and fluorescence correlation spectroscopy (FCS), without a need for sepn. of free and bound label, is disclosed. Nucleic acid amplification is carried out with a pair of **primers**, one **labeled** and the other unlabeled. The **label** is preferably a fluorescent **label**, and unlabeled **primers** are in excess. The method also comprises the steps of obtaining multiple data measurement within a limited 3D visual field on the soln. mobility/fluctuation of the label, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The limited 3D visual field is diffraction limited range formed by confocal optics. Single photon measurement mode is used. The method was applied to the quant. anal. of lambda phage DNA using a rhodamine **labeled primers**.
IT **Primers** (nucleic acid)
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(**labeled**; method and app. for quant. anal. of target nucleic acid by polymerase chain reaction and fluorescence correlation spectroscopy)

L5 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2001 ACS
AN 2000:275403 CAPLUS
DN 132:304266
TI Quantitative determination of target nucleic acid by **asymmetric PCR** and fluorescence correlation spectroscopy
IN Kinjo, Masataka
PA Japan
SO Ger. Offen., 18 pp.
CODEN: GWXXBX
DT Patent
LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 19950823	A1	20000427	DE 1999-19950823	19991021
	JP 2000125900	A2	20000509	JP 1998-301316	19981022
PRAI	JP 1998-301316		19981022		

AB The invention concerns a quant. procedure for target nucleic acid detection using a amplification reaction and a **labeled primer**. No sepn. of free and hybridized labeled DNA is necessary. Thus, a PCR reaction using a non-tagged first primer and a fluorescence-tagged second primer, present in fewer copies than the first primer, is carried out. The reaction may be followed by fluorescence correlation spectroscopy. These results are statistically analyzed and used to det. the no. of target nucleic acids in the sample.

TI Quantitative determination of target nucleic acid by **asymmetric PCR** and fluorescence correlation spectroscopy

AB The invention concerns a quant. procedure for target nucleic acid detection using a amplification reaction and a **labeled primer**. No sepn. of free and hybridized labeled DNA is necessary. Thus, a PCR reaction using a non-tagged first primer and a fluorescence-tagged second primer, present in fewer copies than the first primer, is carried out. The reaction may be followed by fluorescence correlation spectroscopy. These results are statistically analyzed and used to det. the no. of target nucleic acids in the sample.

IT **Primers** (nucleic acid)
RL: ARG (Analytical reagent use); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(fluorophore-**labeled**; quant. detn. of target nucleic acid by asym. PCR and fluorescence correlation spectroscopy)

L5 ANSWER 3 OF 11 MEDLINE DUPLICATE 1
AN 2000107151 MEDLINE
DN 20107151 PubMed ID: 10639495
TI PCR-derived ssDNA probes for fluorescent in situ hybridization to HIV-1 RNA.
AU Knuchel M C; Graf B; Schlaepfer E; Kuster H; Fischer M; Weber R; Cone R W
CS Division of Infectious Diseases, Department of Internal Medicine, University Hospital Zurich, Zurich, Switzerland.
SO JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (2000 Feb) 48 (2) 285-94. Journal code: IDZ; 9815334. ISSN: 0022-1554.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000314
Last Updated on STN: 20000314
Entered Medline: 20000229
AB We developed a simple and rapid technique to synthesize single-stranded DNA (ssDNA) probes for fluorescent in situ hybridization (ISH) to human immunodeficiency virus 1 (HIV-1) RNA. The target HIV-1 regions were amplified by the polymerase chain reaction (PCR) and were simultaneously labeled with dUTP. This product served as template for an optimized **asymmetric PCR** (one-**primer** PCR) that incorporated digoxigenin (dig)-**labeled** dUTP. The input DNA was subsequently digested by uracil DNA glycosylase, leaving intact, single-stranded, digoxigenin-labeled DNA probe. A cocktail of ssDNA probes representing 55% of the HIV-1 genome was hybridized to HIV-1-infected 8E5 T-cells and uninfected H9 T-cells. For comparison, parallel hybridizations were done with a plasmid-derived RNA probe mix covering 85% of the genome and a PCR-derived RNA probe mix covering 63% of the genome. All three probe types produced bright signals, but the best signal-to-noise ratios and the highest sensitivities were obtained with the ssDNA probe. In addition, the ssDNA probe syntheses generated large amounts of probe (0.5

to 1 microg ssDNA probe per synthesis) and were easier to perform than the RNA probe syntheses. These results suggest that ssDNA probes may be preferable to RNA probes for fluorescent ISH. (J Histochem Cytochem 48:285-293, 2000)

AB . . . by the polymerase chain reaction (PCR) and were simultaneously labeled with dUTP. This product served as template for an optimized **asymmetric PCR** (one-**primer** PCR) that incorporated digoxigenin (dig)-**labeled** dUTP. The input DNA was subsequently digested by uracil DNA glycosylase, leaving intact, single-stranded, digoxigenin-labeled DNA probe. A cocktail of. . .

L5 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2000:4853 BIOSIS
DN PREV200000004853
TI Detection of Escherichia coli O157:H7 DNA using two fluorescence polarization methods.
AU Ye, Bang-Ce (1); Ikebukuro, Kazunori; Karube, Isao
CS (1) Key State Laboratory of Bioreactor Engineering, Research Institute of Biochemistry, East China University of Science and Technology, MeiLong Road 130, 200237, Shanghai China
SO Fresenius' Journal of Analytical Chemistry, (Nov., 1999) Vol. 365, No. 5, pp. 452-457.
ISSN: 0937-0633.
DT Article
LA English
SL English
AB Using stx 2 gene in verotoxin-producing Escherichia coli O157:H7 as a target DNA, polymerase chain reaction (PCR) amplification has been combined with fluorescence polarization (FP) by two distinct combination protocols. The first approach (PCR-probe-FP) requires that fluorescence labeled specific probes are hybridized with the **asymmetric PCR** product. In the second protocol (PCR-**primer**-FP), the fluorescence **labeled primer** is used in PCR amplification. In both methods, the PCR products are detected using fluorescence polarization. Hybridization (in the PCR-probe-FP method) and conversion (in the PCR-**primer**-FP method) of 5'-fluorescence **labeled** oligodeoxynucleotide to the PCR product are monitored by an increase in the anisotropy ratio. The results demonstrate the importance of **asymmetric PCR** (in the first method) and the selection of **dye**-modified **primer** concentration (in the second method) for designing a polarization strategy for the detection of DNA sequence. It has been found that the methods can be used for the identification of infectious agents. This system has also been applied to the determination of Escherichia coli O157:H7 strains.

AB. . . (FP) by two distinct combination protocols. The first approach (PCR-probe-FP) requires that fluorescence labeled specific probes are hybridized with the **asymmetric PCR** product. In the second protocol (PCR-**primer**-FP), the fluorescence **labeled primer** is used in PCR amplification. In both methods, the PCR products are detected using fluorescence polarization. Hybridization (in the PCR-probe-FP method) and conversion (in the PCR-**primer**-FP method) of 5'-fluorescence **labeled** oligodeoxynucleotide to the PCR product are monitored by an increase in the anisotropy ratio. The results demonstrate the importance of **asymmetric PCR** (in the first method) and the selection of **dye**-modified **primer** concentration (in the second method) for designing a polarization strategy for the detection of DNA sequence. It has been found. . .

L5 ANSWER 5 OF 11 MEDLINE
AN 2000189615 MEDLINE
DN 20189615 PubMed ID: 10727081
TI DENS (differential extension with nucleotide subsets): application to the sequencing of human genomic DNA and cDNA.

AU Zevin-Sonkin D; Liberzon A; Chochikyan A; Hovhannisyan H; Lvovsky L; Ulanovsky L
CS Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel.
SO DNA SEQUENCE, (1999) 10 (4-5) 245-54.
Journal code: A9H; 9107800. ISSN: 1042-5179.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200004
ED Entered STN: 20000421
Last Updated on STN: 20000421
Entered Medline: 20000412
AB Here we describe further development of our method of DNA sequencing by Differential Extension with Nucleotide Subsets (DENS) and its application to the sequencing of human genomic DNA and full-insert cDNA. Essentially, DENS is primer walking without custom primer synthesis; instead, DENS uses a presynthesized library of octamer primers degenerate in two positions (4,096 tubes/sequences for a complete library). DENS converts an octamer selected from this library into a long primer on the template, at the intended site only. This is done using a two-step procedure which starts with a limited extension of the octamer (at 20 degrees C) in the presence of only two of the four possible dNTPs. The primer is extended by five bases or more at the intended priming site, which is deliberately selected to maximize the extension length (as are the two-dNTP set and the primer itself). The subsequent termination reaction at 60 degrees C then accepts the primer extended at the intended site, but not at alternative sites, where the initial extension (if any) is generally much shorter. This paper presents a set of rules for selection of DENS priming sites. We also compare different ways of template preparation for DENS sequencing. The data were obtained from primer walking on three human genomic DNA subclones of 3 to 4 kbp and four cDNA clones containing inserts of 1.9, 2.3, 3.8, and 4.9 kbp. Full-length sequences were obtained from both strands of each subclone by automated **dye**-terminator fluorescent DNA sequencing using DENS with degenerate octamer **primers**. We compared the following types of DNA templates: single-stranded and double-stranded phagemid DNA, double-stranded PCR products, **asymmetric PCR** products, and single-stranded DNA produced by digestion with Lambda Exonuclease of double-stranded PCR product phosphorylated at one end (Exo-PCR). While all of the preps were found to work, the best results were obtained with Exo-PCR and phagemid single-stranded DNA. Exo-PCR directly from overnight bacterial culture with no plasmid prep of any kind yielded templates for DENS as good as Exo-PCR from purified DNA. We found that the Tm of the differentially extended octamers is an important factor in the success of DENS. Clustering of successful reactions was clearly distinguished in the Tm range of 50-66 degrees C, with success rates of 70% for Exo-PCR and 65% for ss phagemid templates.
AB . . . inserts of 1.9, 2.3, 3.8, and 4.9 kbp. Full-length sequences were obtained from both strands of each subclone by automated **dye** -terminator fluorescent DNA sequencing using DENS with degenerate octamer **primers**. We compared the following types of DNA templates: single-stranded and double-stranded phagemid DNA, double-stranded PCR products, **asymmetric PCR** products, and single-stranded DNA produced by digestion with Lambda Exonuclease of double-stranded PCR product phosphorylated at one end (Exo-PCR). While. . .

L5 ANSWER 6 OF 11 MEDLINE DUPLICATE 2
AN 1999010071 MEDLINE
DN 99010071 PubMed ID: 9793656
TI Detection of **asymmetric PCR** products in homogeneous solution by fluorescence correlation spectroscopy.
AU Kinjo M

CS Laboratory of Biophysics, Hokkaido University, Sapporo, Japan.
SO BIOTECHNIQUES, (1998 Oct) 25 (4) 706-12, 714-5.
Journal code: AN3; 8306785. ISSN: 0736-6205.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199812
ED Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981230
AB The yield of the double-stranded DNA product (500 bp) of **asymmetric PCR** with a rhodamine-**labeled** **primer** (Rho-**primer**) was determined in a homogeneous solution using fluorescence correlation spectroscopy (FCS). FCS provides the average number of molecules in a focused volume and the diffusion constant that relates the molecular weight. Since FCS measures the fluctuation of fluorescence intensity in a very small sample volume, the reaction mixture was directly placed on the FCS optical field without any purification procedure after amplification. The result of changing the initial number of templates suggested that elongation of the Rho-primer could be detected by FCS in a PCR mixture containing a single copy of the target gene in the initial condition. Possible scientific applications and perspectives of the proposed approach are discussed.
TI Detection of **asymmetric PCR** products in homogeneous solution by fluorescence correlation spectroscopy.
AB The yield of the double-stranded DNA product (500 bp) of **asymmetric PCR** with a rhodamine-**labeled** **primer** (Rho-**primer**) was determined in a homogeneous solution using fluorescence correlation spectroscopy (FCS). FCS provides the average number of molecules in a. . .

L5 ANSWER 7 OF 11 MEDLINE DUPLICATE 3
AN 1998110314 MEDLINE
DN 98110314 PubMed ID: 9448847
TI Integrated amplification and detection of the C677T point mutation in the methylenetetrahydrofolate reductase gene by fluorescence resonance energy transfer and probe melting curves.
AU Bernard P S; Lay M J; Wittwer C T
CS Department of Pathology, University of Utah Medical School, Salt Lake City 84132, USA.
SO ANALYTICAL BIOCHEMISTRY, (1998 Jan 1) 255 (1) 101-7.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199803
ED Entered STN: 19980410
Last Updated on STN: 19980410
Entered Medline: 19980327
AB A microvolume fluorimeter integrated with a rapid thermal cycler allows both amplification and point mutation detection from genomic DNA in approximately 30 min. This homogeneous method combines rapid cycle DNA amplification with allele-specific fluorescent probe melting profiles for product genotyping. The amplification reaction includes a **primer** internally **labeled** with Cy5 and a 3'-fluorescein-**labeled** probe that spans the region of interest. During **asymmetric** **amplification**, the probe hybridizes to excess Cy5-labeled strand and is observed as fluorescence resonance energy transfer. Resonance energy transfer increases each cycle as product accumulates during amplification. When fluorescence is monitored as the temperature increases through the Tm of the probe/product duplex, a characteristic melting profile for each genotype is obtained. Fluorescence genotyping of the

common C677T base substitution in the methylenetetrahydrofolate reductase gene in 110 DNA samples correlated perfectly with genotyping by restriction enzyme digestion and gel electrophoresis. The relatively stable G:T mismatch of this example gave a 3 degrees C difference in Tm from complete Watson-Crick pairing, suggesting that this homogeneous fluorescence method can be used for all single-base mismatches.

AB . . . method combines rapid cycle DNA amplification with allele-specific fluorescent probe melting profiles for product genotyping. The amplification reaction includes a **primer** internally **labeled** with Cy5 and a 3'-fluorescein-**labeled** probe that spans the region of interest. During **asymmetric amplification**, the probe hybridizes to excess Cy5-labeled strand and is observed as fluorescence resonance energy transfer. Resonance energy transfer increases each. . .

L5 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2001 ACS
AN 1993:574886 CAPLUS
DN 119:174886
TI Rapid sequencing of unpurified PCR products by thermal **asymmetric PCR** cycle sequencing using unlabeled sequencing primers
AU Liu, Yao Guang; Mitsukawa, Norihiro; Whittier, Robert F.
CS Mitsui Plant Biotechnol. Res. Inst., RITE, Tsukuba, 305, Japan
SO Nucleic Acids Res. (1993), 21(14), 3333-4
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB A new sequencing strategy which omits PCR product purifn., yet does not require end-**labeled primers** for generating clear sequencing ladders is reported. The strategy relies on thermal asym. PCR and utilizes one or two relatively short primers in the initial PCR amplification of target DNA. Subsequent sequencing is carried out by temp. cycling using a longer sequencing primer. Sequencing cycles employ a high annealing temp. to avoid annealing and elongation of carried-over short primers. Sequencing by this strategy is reproducible and reliable without requiring reoptimization of conditions for each new PCR product, and the sequencing quality is as good as with purified samples. In most cases sequence detn. is limited only by the resolving power of the gel.
TI Rapid sequencing of unpurified PCR products by thermal **asymmetric PCR** cycle sequencing using unlabeled sequencing primers
AB A new sequencing strategy which omits PCR product purifn., yet does not require end-**labeled primers** for generating clear sequencing ladders is reported. The strategy relies on thermal asym. PCR and utilizes one or two relatively short primers in the initial PCR amplification of target DNA. Subsequent sequencing is carried out by temp. cycling using a longer sequencing primer. Sequencing cycles employ a high annealing temp. to avoid annealing and elongation of carried-over short primers. Sequencing by this strategy is reproducible and reliable without requiring reoptimization of conditions for each new PCR product, and the sequencing quality is as good as with purified samples. In most cases sequence detn. is limited only by the resolving power of the gel.

L5 ANSWER 9 OF 11 MEDLINE DUPLICATE 4
AN 92225344 MEDLINE
DN 92225344 PubMed ID: 1563631
TI A rapid polymerase-chain-reaction-directed sequencing strategy using a thermostable DNA polymerase from Thermus flavus.
AU Rao V B; Saunders N B
CS Department of Biology, Catholic University of America, Washington, DC 20064.
SO GENE, (1992 Apr 1) 113 (1) 17-23.
Journal code: FOP; 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals
EM 199205
ED Entered STN: 19920607
Last Updated on STN: 19980206
Entered Medline: 19920520
AB We have developed a polymerase chain reaction (PCR)-directed sequencing strategy for rapid sequencing of DNA from crude viral or cell preparations. The basic strategy consists of two phases. In the first phase, the target DNA is amplified by symmetric PCR with low concentrations of deoxyribonucleotide triphosphate (dNTP) and oligodeoxyribonucleotide primers. This results in exponential amplification of DNA in the initial cycles, reaching a plateau by 25 cycles due to limiting concentrations of dNTP and primers. In the second phase, a small aliquot of the PCR mixture is amplified without any purification, by **asymmetric PCR** in the presence of a 5'-**labeled primer** and one of the four dideoxyribonucleotide triphosphates. This results in the accumulation of single-stranded DNA products that are terminated at specific points by incorporation of the appropriate dideoxyribonucleotide monophosphate. The products are then analyzed by electrophoresis on a sequencing gel followed by autoradiography. The PCR conditions are optimized to generate sequence ladders of several hundred nucleotides starting from as low as 100 copies of bacteriophage or bacterial genome in one to two days.
AB . . . dNTP and primers. In the second phase, a small aliquot of the PCR mixture is amplified without any purification, by **asymmetric PCR** in the presence of a 5'-**labeled primer** and one of the four dideoxyribonucleotide triphosphates. This results in the accumulation of single-stranded DNA products that are terminated at. . .

L5 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1992:429037 BIOSIS
DN BA94:81162
TI HIGH-THROUGHPUT FLUORESCENT DNA SEQUENCE ANALYSIS METHODS AND AUTOMATION.
AU WILSON R K; HOOD L
CS DEP. GENET., WASHINGTON UNIV. SCH. MED., 660 S. EUCLID AVE., ST. LOUIS, MO. 63110, USA.
SO METHODS (ORLANDO), (1991) 3 (1), 48-54.
CODEN: MTHDE9. ISSN: 1046-2023.
FS BA; OLD
LA English
AB A strategy for high-throughput DNA sequence analysis using the fluorescent **dye-primer** chemistry is described. This strategy combines rapid preparation of template DNA using a modification of the polymerase chaini reaction (PCR), automation of the DNA sequencing reactions using a robotic laboratory workstation, and subsequent analysis of the fluorescent-labeled reaction products on a commercial automated fluorescent sequencer. **Asymmetric PCR** provides a reliable means of simultaneously producing sufficient and uniform quantities of several template DNAs directly from bacterial colonies or bacteriophage plaques. The automation scheme for subsequent processing and analysis of DNA samples allows a DNA sequencing facility to operate the fluorescent sequencer twice daily at its full capacity of 24 samples. Furthermore, the methods and instruments described eliminate much of the labor required by technicians to obtain a high level of data output. As described here, a DNA sequencing laboratory equipped with a single automated fluorescent sequencer can perform analysis of up to 48 fluorescent-labeled reactions every day, with an average output of over 10,000 bp per sequence run.
AB A strategy for high-throughput DNA sequence analysis using the fluorescent **dye-primer** chemistry is described. This strategy combines rapid preparation of template DNA using a modification of the polymerase chaini reaction (PCR),. . . reactions using a robotic laboratory workstation, and subsequent analysis of the fluorescent-labeled reaction products on a commercial automated fluorescent sequencer.

Asymmetric PCR provides a reliable means of
simultaneously producing sufficient and uniform quantities of several
template DNAs directly from bacterial colonies or. . .

L5 ANSWER 11 OF 11 MEDLINE
AN 90211914 MEDLINE
DN 90211914 PubMed ID: 2483662
TI Optimization strategies for the polymerase chain reaction.
AU Williams J F
CS Perkin-Elmer Corporation, Norwalk, CT 06859-0251.
SO BIOTECHNIQUES, (1989 Jul-Aug) 7 (7) 762-9.
Journal code: AN3; 8306785. ISSN: 0736-6205.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199005
ED Entered STN: 19900622
Last Updated on STN: 19980206
Entered Medline: 19900514
AB The GeneAmp polymerase chain reaction (PCR) process has now become a key procedure in molecular biology research laboratories. The PCR technique is an in vitro method in which genomic or cloned target sequences are specifically enzymatically amplified as directed by a pair of oligonucleotide primers. This technique has been quite robust in the hands of the majority of researchers and is extremely flexible, as evidenced by the increasing number of related PCR formats (i.e., inverse PCR, anchored PCR, **asymmetric PCR**, **labeled primer**
PCR and RNA-PCR). Today's applications include direct sequencing, genomic cloning, DNA typing, detection of infectious microorganisms, site-directed mutagenesis, prenatal genetic disease research, and analysis of allelic sequence variations. Scientists at Cetus and Perkin-Elmer have collaborated for several years to better understand the interacting biochemical and biophysical parameters which affect PCR optimization. Following are many of the current recommendations, offered with the caveat that our understanding of the PCR process is continually evolving.
AB . . . researchers and is extremely flexible, as evidenced by the increasing number of related PCR formats (i.e., inverse PCR, anchored PCR, **asymmetric PCR**, **labeled primer** PCR
and RNA-PCR). Today's applications include direct sequencing, genomic cloning, DNA typing, detection of infectious microorganisms, site-directed mutagenesis, prenatal genetic. . .

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